GC/MS and EMIT Analyses for Δ^9-Tetrahydrocannabinol Metabolites in Plasma and Urine of Human Subjects*

L.J. McBurney, B.A. Bobbie***, and L.A. Sepp**

Defense and Civil Institute of Environmental Medicine, 1133 Sheppard Avenue West, P.O. Box 2000, Downsview, Ontario, Canada M3M 3B9

UNLIMITED
DISTRIBUTION
ILLIMITÉE

Abstract

Ten male subjects smoked placebo marijuana cigarettes spiked with Δ^9-tetrahydrocannabinol (THC) at 150 μg/kg body weight. Plasma and urine samples were collected for 22 hr after smoking. Total cross-reacting cannabinoids were measured by immunoassay (EMIT) and THC and major metabolites were identified and quantitated by gas chromatographic/mass spectrometric (GC/MS) procedures. In plasma, THC concentration provided the best indication of recent (<6 hr) smoking. In enzyme-hydrolyzed urine, 8β, 11-dihydroxy-THC at high concentration was identified in the earliest voidings, falling rapidly to the limit of detection before 22 hr in most subjects. Levels above 15 to 20 ng/mL were indicative of use within the previous 4 to 6 hr.

Introduction

The detection of marijuana use by immunoassay drug screening procedures for cannabinoids in human body fluids is now widely used (1-5). Because of the potential for false positive results and for cross-reactivity of antibody with a broad spectrum of cannabinoids in biological samples, such methods may not be considered specific for Δ^9-tetrahydrocannabinol (THC) or its metabolites. For forensic purposes, positive results must be validated by other more specific methods. Gas chromatography/mass spectrometry (GC/MS) is frequently recommended and used for confirmation of the results obtained by immunoassay techniques, and to generate quantitative data for metabolite profiling in clinical studies on marijuana use (5,6). For medicolegal purposes, the demonstration of recent marijuana usage through analysis of THC and its metabolites in human biological specimens is receiving increasing attention (7-9). It has been shown frequently that 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) is the major metabolite in both blood and urine (10-13). However, as indicated by the persistence of measurable cannabinoid levels using immunoassays most sensitive to THC-COOH (2,9), this metabolite may be present for days in blood and for weeks in urine after cessation of smoking or ingestion. Its presence, therefore, may merely indicate exposure to marijuana at some previous indeterminate time. A number of investigators have examined THC levels in blood and THC-COOH levels, both free and glucuronide-bound, in urine following marijuana use. Their results have suggested that ratios such as free/bound THC-COOH in urine or total cannabinoid/THC in plasma may be related to elapsed time since intake (7,10,14,15).

The parent THC is almost completely metabolized in the body and unchanged THC is not seen in urine in sufficient amounts to be readily quantitated (16). The major proportion of urinary metabolites are highly polar acids, with the single most abundant acid, THC-COOH, accounting for about 27% of excreted metabolites (11). Some 17 other carboxylic and dicarboxylic acids have been identified as minor metabolites in human urine (17,18). Because of the non-invasive nature of urinalysis for drug detection it would be desirable to find one or more early-occurring urinary metabolites with short half-lives whose presence in urine would indicate quite recent drug use. In an earlier study, a dihydroxy-THC metabolite was seen in the urine of several marijuana smokers shortly after smoking but not the following day (19). Such a metabolite shows promise as a potential indicator of recent cannabis use.

This paper reports the analytical results obtained for total cannabinoids, THC, and THC metabolites in plasma and urine collected for 22 hr after smoking a standardized dose of THC. These results form part of a larger study carried out jointly with the Road Safety Unit of Ministry of Transport Canada. In this study psychomotor tasks were performed and breath samples were also taken. Preliminary results concerned with the relationship between plasma THC levels and performance on a laboratory task, and THC in alveolar air, have been reported elsewhere (20,21).

Methods

All experimentation with human subjects was conducted in accordance with a protocol approved by the DCIEM Human Ethics Committee and by the Health Protection Branch of Health and Welfare Canada. Placebo cannabis, from which can-

*DCIEM Research Paper #85-P-44. Presented in part at the 13th Scientific Session of the Joint Committee on Aviation Pathology, Toronto, 1982.

**Author to whom correspondence should be addressed.

***Present address: Ontario Ministry of the Environment, Rexdale, Ontario.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

nabinoids had been extracted, and a THC standard used in this study were supplied by the Scientific Services Division, Health and Welfare Canada. Cannabinoid standards 11-hydroxy-THC (11-OH-THC), THC-COOH, deuterated THC-COOH (THC-COOH-d_3), 8α,11-dihydroxy-THC (8α,11-diOH-THC), and 8β,11-diOH-THC (diOH-THC) were also supplied by Health and Welfare Canada.

Subject Selection

The subjects for this study were 10 male volunteers between the ages of 18 and 30. All were experienced occasional social users familiar with the effects of marijuana. Each subject underwent medical screening to ensure that his body fat was between 13 and 25%, that he was not currently using any psychoactive drugs, and that he did not exhibit any gross pathology. Subjects were requested not to smoke marijuana for the week preceding each experimental session. Sessions were spaced one week apart to permit clearance of significant levels of the drug from the body. On each occasion a heparin-lock cannula (Butterfly-19, Abbott Laboratories Ltd.) was inserted into a vein on the back of one forearm and a preliminary plasma sample taken. Subjects were requested to void their bladders and supply a urine sample.

Drug Administration

Each subject was required to smoke two cannabis cigarettes over a 30-min period during each of two sessions. The two sessions were identical except that placebo cannabis spiked with THC was administered during one and placebo cannabis without THC during the other. The spiked cannabis cigarettes were prepared individually for each subject to ensure a presented dose of 150 μg THC/kg of body weight divided into two cigarettes of equal weight. Unspiked cigarettes contained the same quantity of placebo cannabis. Subjects required from 11 to 14 min to finish each cigarette and the interval between cigarettes was adjusted so that a total smoking time of 30 min was maintained by each individual. Cigarette butts were not analyzed for residual THC content.

Sample Collection

Subjects provided 7-mL blood samples via the indwelling venous catheter at 5, 10, 20, 30, and 50 min and at 1, 2, 3, 4, and 5 hr after smoking. A final blood sample was collected at 22 hr by separate venipuncture. Plasma was separated immediately and frozen in silanized glass vials prior to analysis. Total urine output was measured and samples of each voiding were saved in silanized glass jars for analysis.

Plasma Cannabinoid Extraction

For GC/MS analysis, plasmas were extracted essentially using the method developed by Rosenfeld (22) with the following modifications incorporated to enable the extraction of THC-COOH. One milliliter of plasma was incubated overnight with 4000 units of β-glucuronidase (Sigma Chemical Co., G 0751), acidified with 1 mL KCl/HCl buffer, pH 2.3, prior to extraction with two 4-mL volumes of Brodie's solvent (1.5% isoamyl alcohol in heptane). Acetone (½ mL) was added to each 4 mL solvent to reduce emulsions. Final extracts were dried in reactivials under a stream of N_2 and stored frozen until GC/MS analysis. Negative plasmas were spiked with varying levels of cannabinoid standards from 3 to 120 ng/mL to determine recoveries over this range of concentration. Plasmas spiked with THC, 11-OH-THC, and THC-COOH at 30 or 3 ng/mL, were included in the procedure each time to monitor recoveries and day-to-day precision.

Urine Cannabinoid Extraction

The extraction procedure used for the GC/MS analysis of urinary cannabinoids is a modification of the method of Foltz and Hidy (23) for the simultaneous analysis of THC, 11-OH-THC, and THC-COOH in plasma. The following modifications were made to permit an enzyme hydrolysis step prior to extraction of cannabinoids. Urine (1 mL) was adjusted to pH 5.3 to 5.4 with 0.2*N* HCl if the pH was above 5.8. One milliliter of 1*M* KCl and 4000 U β-glucuronidase were added and samples incubated overnight or for a minimum of 5 hr at 37°C with gentle shaking in a Dubnoff shaker. Following enzyme hydrolysis, samples were placed in a boiling water bath for 1 min to denature the enzyme. Contents were buffered to pH 2 to 3 by the addition of 0.06 mL 1*N* HCl and extracted twice with 4-mL volumes of hexane:ethyl acetate (7:1). Subsequent extraction procedure was as per Foltz and Hidy (23).

Negative urines were spiked with varying levels of THC-COOH and diOH-THC from 3 to 120 ng/mL to determine recoveries and provide calibration curves for quantitative measurements. Urines spiked with 50 ng/mL THC-COOH, 30 ng/mL diOH-THC, and 10 ng/mL THC were carried through the extraction procedure each time to monitor daily recoveries and precision. When it became available for the study, the deuterated acid metabolite THC-COOH-d_3 was also used for control urines as an internal standard and results compared to an external standard calibration curve for THC-COOH.

Derivatization and GC/MS Analysis of Cannabinoids

The dried extracts were derivatized in a single step using the procedure developed by Hazan et. al. (19) for cannabinoids in urine. Hexane (100 μL) (Burdick and Jackson), 50 μL PFPA (pentafluoropropionic anhydride), (Chromatographic Specialties), and 50 μL HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) (Fisher Scientific) were added to each extract. Reactivials were heated for 10 min at 100°C. PFPA derivatizes the alcohol and/or phenolic moiety and HFIP converts the carboxylic acid function to the HFIP ester. After cooling and evaporation of the derivatizing reagents under nitrogen, each residue was dissolved in 20 or 25 μL chloroform (Burdick and Jackson).

Mass spectrometric measurements were performed on a model 4021 GC/MS (Finnigan) interfaced to an INCOS data system. The MS was operated in the EI mode. The GC was equipped with a 40-m x 0.5-mm i.d. support-coated open tubular (SCOT) capillary column containing 3% OV-17 on Chromosorb W (G-SCOT/B/OV-17, Chromographic Specialties) and was run isothermally at 225°C with helium as the carrier gas. The capillary column was interfaced directly to the MS source by means of a short length of fused silica DB-54 column. Injector, interface, and ion source temperatures were 250°, 260°, and 270°C, respectively. The ionization energy was set to 60eV. Scan rates for full scans or selected multiple ion detection (MID) were 1 scan/sec. For quantitation, at least three major diagnostic ions for each derivatized cannabinoid were monitored.

SCOT columns are no longer readily available, having been replaced by WCOT or FSOT capillary columns. The authors currently use an FSOT 30-m x .25-mm DB-17 column (J&W), 0.25-μ film thickness, which provides very similar results to those reported in this study.

EMIT-d.a.u. Cannabinoid Analyses

Total cannabinoid analyses in urine samples were carried out using reagent kits, calibrator kits, and instructions (24) as supplied by Syva Co. (3M019, 3M109, Cannabinoid Assay G6PDH). In addition to the urine calibrators supplied by Syva (cut-off 20 ng/mL, medium 75 ng/mL), negative urines were spiked with THC-COOH at levels from 5 to 100 ng/mL to provide a standard curve for quantitation over a broader range than that covered by the manufacturer's calibrators.

For total cannabinoid analysis in plasma, samples were extracted using a procedure developed for whole blood (25,26). For these methanolic extracts, the reading time was increased to 55 sec from the 30-sec reaction time recommended for urine analysis. Negative plasmas were spiked with THC-COOH at levels from 5 to 100 ng/mL and carried through the procedure at the same time to prepare a calibration curve for quantitation of total cannabinoids recovered.

Results and Discussion

GC/MS

The mass spectra generated for derivatized cannabinoid standards used in this study are shown in Figure 1. Diagnostic ions (m/z) used for verification of presence and for quantitation of each compound are listed in Table I with percentage relative abundance for each m/z shown in parentheses.

The chromatogram of the 8β,11-diOH-THC standard derivatized with PFPA exhibited three peaks; two had the correct molecular weight for a diOH-THC of 784, while the third was identified as cannabinol on the basis of its mass spectrum and retention time. Blank urines spiked with 8β,11-diOH-THC and carried through the extraction procedure also displayed the two diOH-THC peaks. In the known positive urine samples, however, only one of the two diOH-THC peaks, designated as peak #1, was seen. This indicated that the double peaks seen in the chromatogram of the reference standard were not due to the extraction/derivatization procedure, but probably reflected a mixture of two isomeric metabolites. Under the present analytical conditions, peak #1 contributed 30% of the total ion count to the 8β,11-diOH-THC standard and for purposes of quantitation a 30% purity was therefore assumed. In an attempt to identify the two peaks of the standard, 8α,11-diOH-THC was examined. This standard also formed three derivatives; two had a molecular weight of 784, but neither corresponded to either peak seen in the chromatogram of the 8β,11-diOH-THC standard (Table I).

Each standard, when derivatized with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) in pyridine (Chromatographic Specialties) for 1 hr at 100°C, displayed a single trimethylsilyl (TMS) derivative with mass spectra identical to those reported in the literature for these metabolites (27-29). The base peaks and diagnostic ions for the TMS derivatives are listed in Table I. These two derivatives were well separated by GC, having retention times of approximately 9 min for 8α,11-diOH-TMS and 11 min for 8β,11-diOH-TMS under these GC/MS conditions. Several subject urines displaying the highest diOH-THC levels were also analyzed after derivatization by BSTFA in pyridine. Only one peak was observed as having the correct molecular weight for a diOH-THC-TMS, and the mass spectrum and retention time corresponded exactly to that for 8β,11-diOH-THC-TMS. TMS derivatization was not otherwise

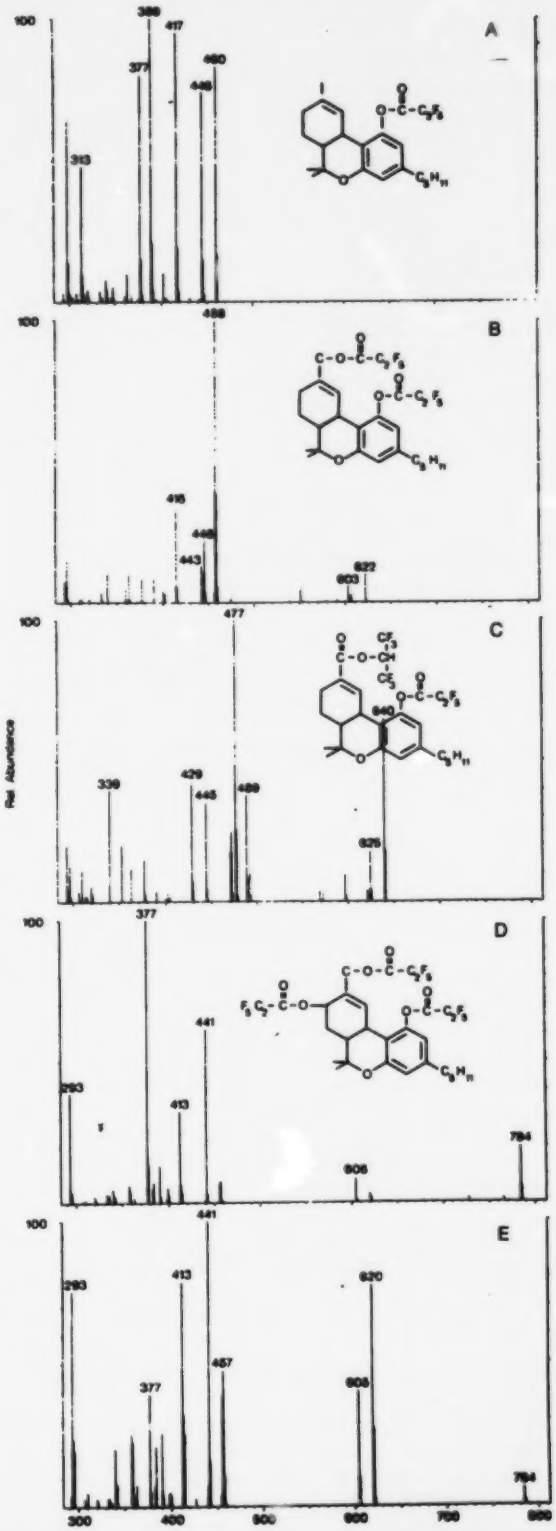

Figure 1. EI mass spectra of PFPA/HFIP derivatives of (A) THC; (B) 11-OH-THC; (C) THC-COOH; (D) 8β,11-diOH-THC, peak #1; and (E) 8β,11-diOH-THC, peak #2.

used in this study for two reasons. These derivatives in the biological extracts were quite unstable compared to the PFPA or PFPA/HFIP derivatives. Also, TMS derivatization gave rise to considerable background interference from co-eluting constituents in the biological matrices.

Both 8α-OH-THC and 8β-OH-THC have been found in human plasma and liver as well as 8α,11-diOH-THC, 8β,11-diOH-THC, and dihydroxy metabolites with a sidechain hydroxyl (28,30). While aliphatic hydroxylations are minor pathways in man, of the allylic alcohols at the 8-position, β-hydroxylation appears to be the more common pathway (30) and would support the identification of 8β,11-diOH-THC as the diOH-THC found in the urine of the subjects. The authors have no explanation as to why the diOH-THC standards formed several peaks when derivatized with PFPA/HFIP whereas only one major peak (purity < 90%) was observed when BSTFA was the derivatizing agent.

Extraction and Recovery of Cannabinoids

In order to extract the acid metabolite from plasma using the modified Rosenfeld extraction procedure, it was necessary to drop the pH below 5. The addition of the enzyme hydrolysis step to the method was also found to be necessary for the recovery of this metabolite and, if omitted, recovery of added THC-COOH fell to less than 10% in spiked plasma samples. The authors cannot readily explain why the addition of enzyme facilitated recovery of this metabolite, especially in the case of added free THC-COOH in the spiked samples. However, Foltz et al. (10) reported that if cannabinoids in plasma are first separated from proteinaceous material using a polar water-miscible solvent such as acetonitrile, they can be subsequently extracted into organic solvents with far greater reproducibility and efficiency than that found with solvent extraction alone. The extraction method adapted for the present study did not include any preliminary deproteinizing step. It can only be conjectured that the added enzyme may have limited or inhibited some non-specific protein binding of free THC-COOH and permitted greater extraction of this metabolite by the extracting solvent (1.5% isoamyl alcohol in heptane) used here. It is also unknown as to whether the enzyme was in fact effective in hydrolyzing any conjugated metabolite which might have been present in the plasma samples. No THC-COOH-glucuronide standard was available to investigate this process in samples spiked with conjugated cannabinoid. Published data on the levels of free vs. conjugated THC-COOH, however, would indicate that after inhalation or intravenous administration of THC, conjugated cannabinoids in plasma are absent or minimal (12,31). After oral ingestion of THC, however, conjugated cannabinoids in plasma are reported variously to occur in small quantities (31) or, more recently, THC-COOH-glucuronide has been found to constitute the major plasma metabolite 4 hr after ingestion (9). Levels of conjugated metabolites in plasma after oral consumption of THC may be quantitatively much more significant than after smoking.

Plasma samples were examined for the presence of any diOH-THC metabolite but none was measurable by the present procedure. Background interference in plasma extracts occurred at the retention time of diOH-THC in sufficient quantity to mask low levels (< 5 ng/mL) of this metabolite if present. Recoveries of cannabinoid standards from plasma spiked with 30 ng/mL of each standard were as follows: 64.9% for THC (CV 10.1%, n = 49), 68.9% for 11-OH-THC (CV 10.9%, n = 49), and 81.7% for THC-COOH (CV 8.9%, n = 46). At 3 ng/mL, recoveries were 90 to 92% of those at 30 ng/mL. At 120 ng/mL, recoveries were 8 to 11% higher than at 30 ng/mL. The minimum detectable level of each cannabinoid was 200 pg per injection, taking a signal-to-background noise ratio of 3:1 as acceptable. This corresponded to 800 pg/mL plasma.

In the urinary extraction of cannabinoids, the extraction procedure of Foltz and Hidy (23) provided higher recoveries than that of Rosenfeld (22), while the reverse was true for plasma analysis. The enzyme hydrolysis step was essential in order to extract the diOH-metabolite from urine. Without enzyme hydrolysis, amounts of diOH-THC were too low to quantitate, indicating that most of this metabolite is excreted complexed to glucuronide or sulphate. Levels of the THC-COOH extracted from subject urines following hydrolysis were more than doubled on the average compared to those seen when the hydrolysis step was omitted. This result is supported by the reports of other investigators that most THC-COOH in urine is present as the glucuronide conjugate (10,12,13). Boiling the enzyme hydrolysates for 1 min to denature the protein following incubation was also found to increase the recovery of all cannabinoids, endogenous and exogenous, by approximately 5%. Boiling, possibly, may have reduced some non-specific protein binding or otherwise exerted some additional hydrolytic action. Recoveries reached a maximum at 5-hr incubation time, but it was more convenient to hydrolyze overnight in order to complete analysis the following day. Overall recoveries using this procedure were as follows: 72.8% for THC-COOH (CV 10.0% at 50 ng/mL, n = 32) and 70.9% for 8β,11-diOH-THC (CV 7.3% at 30 ng/mL, n = 32). At 3 ng/mL, recoveries were 65.5% for THC-COOH (CV 13.9%) and 64.1% for diOH-THC (CV 10.2%), while at 120 ng/mL, they were 76.1% (CV 4.4%) and

Table I. Diagnostic Ions Used For Monitoring Cannabinoids in Biological Fluids*

Cannabinoid	RRT	Molecular Weight	m/z**	m/z	m/z	m/z
Δ^9-THC/PFPA	1.000	460(83)	377(80)	389(100)	445(74)	—
11-OH-Δ^9-THC/PFPA	1.432	622(10)	415(34)	443(13)	458(100)	—
11-nor-Δ^9-THC-COOH/PFPA/HFIP	1.151	640(65)	445(35)	477(100)	489(38)	625(18)
11-nor-Δ^9-THC-COOH-d_3/PFPA/HFIP	1.146	643(63)	448(36)	480(100)	492(38)	628(16)
8β,11-diOH-THC/PFPA (peak #1 urine/std)	0.493	784(20)	377(100)	413(32)	441(60)	620(3)
8β,11-diOH-THC/PFPA (peak #2, std only)	0.614	784(6)	377(40)	413(80)	441(100)	620(77)
8α,11-diOH-THC/PFPA (peak #1)	0.514	784(2)	339(93)	415(100)	459(20)	579(23)
8α,11-diOH-THC/PFPA (peak #2)	0.706	784(2)	358(24)	413(14)	441(100)	605(32)
8α,11-diOH-THC/TMS	1.769	562(0)	369(12)	457(12)	472(100)	—
8β,11-diOH-THC/TMS	2.193	562(14)	369(100)	459(43)	472(12)	—

*Column used was a 40-m x 0.5-mm i.d. SCOT capillary packed with 3% OV-17 on Chromosorb W, run isothermally at 225°C with 18 psig helium as carrier gas. Ionization energy was 60eV.
**Numbers in parentheses are percentage relative abundances for masses above m/z 300.

73.8% (CV 4.4%), respectively. The limits of detection were 500 pg/mL for THC-COOH and 800 pg/mL for diOH-THC at a signal-to-noise ratio of 3:1.

EMIT-d.a.u. Cannabinoid Analyses

Negative plasmas spiked with THC-COOH at levels from 5 to 100 ng/mL provided the standard curve, and total cannabinoids in subject plasmas were quantified by interpolation using this curve. A reaction reading time of 55 sec gave approximately the same response (change in absorbance, ΔA) for plasma containing 75 ng/mL as the medium calibrator for urine analysis gave over the standard 30-sec reaction time. The longer reaction time of 80 sec used in whole blood analysis (26) in this experience resulted in wider relative variation in absorbance shifts for repetitive readings and hence somewhat poorer precision. A similar finding was reported for whole blood analyses when the effect on absorbance of a 120-sec reaction time was compared with the 80-sec reaction time chosen in that study (26).

Subject Plasma Cannabinoid Levels

The mean plasma profiles of THC and two metabolites by GC/MS, and of total cross-reactive cannabinoids by EMIT are illustrated in Figure 2. Further information on plasma levels for each of the 10 subjects is provided in Table II. Subject #2 was deemed non-compliant since his pre-smoking plasma was found to contain 37 ng/mL total cannabinoid by EMIT, although levels of THC, 11-OH-THC, and THC-COOH, if present, were below the level of sensitivity for GS/MS confirmation. The inter-individual variation in THC levels immediately after smoking was very broad with almost a five-fold difference between lowest and highest values observed for the subject group. Such wide variation is expected because of individual differences in smoking experience and technique. This has been noted previously in clinical smoking studies (32-35). In general, the mean plasma levels of THC agreed quite well with those reported in other studies where approximately equivalent amounts of THC were smoked in controlled clinical situations (32,33,35-37). Also, the mean levels of plasma THC-COOH were in close agreement with the published data in the few studies in which plasma or serum levels of THC-COOH are measured as well (7,12,37).

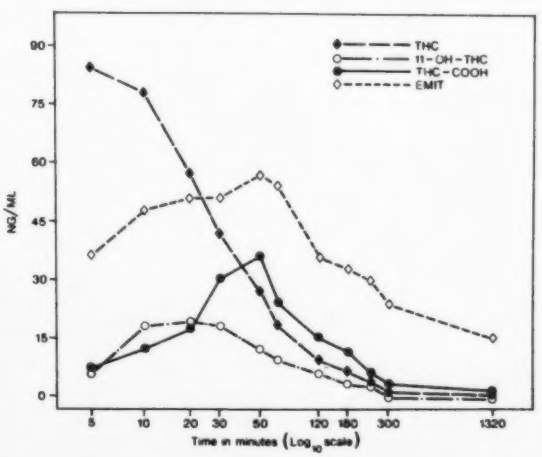

Figure 2. Mean plasma cannabinoid levels by GC/MS and EMIT.

In these investigations, THC-COOH levels averaged 1.7 to 4.5 times the THC levels found from about 1 hr post-smoking on. This compares fairly closely to the average THC-COOH:THC ratios of 1.3 to 3.4 observed from 1 to 5 hr post-smoking in this study. The analytical methods used in these studies would be expected to report total THC-COOH in plasma or serum. Radioimmunoassay techniques such as those employed by Perez-Reyes et al. (37) and Hanson et al. (7) are cross-reactive to both free and bound forms of the acid metabolite, and Wall and Perez-Reyes (12) found minimal conjugated cannabinoids by their procedure.

There was good correlation between the mean EMIT concentrations and totals of mean THC-COOH plus 11-OH-THC with a correlation coefficient (r) of 0.946 (n = 11). However, for individual paired values the correlation was very poor (r = 0.267, n = 96). Differences in individual metabolism of THC with varying proportions of other metabolites produced, as well as differing degrees of glucuronide conjugate formation could account for the lack of correlation. Immunoassays used for screening are at best only semi-quantitative, since most do not measure a single compound but a number of related compounds with widely varying affinities (3,8,38,39). EMIT values in this study were obtained after smoking THC only and therefore represent a response limited to THC metabolites. It would be reasonable to expect that after smoking marijuana containing a broad spectrum of cannabinoids, EMIT would correlate even less effectively to individual THC metabolites.

Plasma metabolite:THC and metabolite:metabolite ratios were examined to determine if any analytical parameter could be found which would correlate with recent smoking. Such a relationship has been suggested before for the ratio of the THC-COOH concentration to the THC concentration in blood and blood serum with time (7). Indeed, in the present study the best correlation was found for log [THC-COOH/THC] vs. log [time post-smoking] and was derived using individual paired values (Figure 3). However, the ratio variability was too broad for this function to be useful on its own for any interpretation concerning time since ingestion. The best indicator of recent use was the level of THC itself. Even in the most efficient smokers' plasmas (subjects 8 to 10), which contained THC levels in excess of 100 ng/mL at the earliest sampling times, these levels had fallen to 2 to 3 ng/mL by 4 to 5 hr. It should be noted, however, that the levels of THC and its metabolites found in plasma after oral ingestion of THC may be expected to differ considerably from those seen after inhalation of THC. Studies comparing oral, intravenous, and inhalation routes of administration show marked differences in metabolism and pharmacokinetics of THC for the oral route as compared to intravenous or inhalation routes. Maximum THC levels after oral ingestion are very much lower, and THC-COOH and 11-OH-THC relatively much higher than those levels seen after smoking or intravenous administration (31,36,40,41).

Subject Urine Cannabinoid Levels

The mean urine profiles of THC-COOH and diOH-THC by GS/MS and of total cross-reacting cannabinoids by EMIT are illustrated in Figure 4. Further information for each of the 10 subjects is provided in Table III. Urines were grouped according to voiding time into five post-smoking time ranges of approximately equal n for tabulation and statistical purposes. Analysis of pre-smoking urines confirmed the presence of THC-COOH for subject #2 at a concentration of 9.9 ng/mL. No diOH-THC was found in any preliminary urine. The maximum levels of

THC-COOH in the subject urines varied by more than 20-fold, ranging from 5.8 to 128 ng/mL. One other study, which followed urinary THC-COOH in occasional marijuana users after smoking a single cigarette containing 19.8 mg THC, reported maximum levels of THC-COOH ranging from 7 to 60 ng/mL, occurring from 2 to 4 hr post-smoking (42). Such wide variation in levels of metabolite excreted is probably a reflection both of differences in smoking efficiency and of inter-individual differences in metabolism. Levels of THC-COOH in chronic smokers, of course, could be substantially higher than those seen in the occasional user. After oral ingestion, also, the urinary excretion of THC-COOH may be higher. Over 90% of an oral dose is absorbed from the gastrointestinal tract (40). Recently Law et al. have reported high levels of cross-reacting cannabinoids in urine (185 to 1063 ng/mL) 6 hr after oral ingestion of 20 mg THC, and positive results for at least a further 12 days (9).

Using the EMIT cutoff value of 20 ng/mL, 10 of the 49 post-smoking urines would be called either negative or borderline positive (Table III). Others also have reported the occurrence of false negatives with EMIT (43). In all 10 cases noted here, urine appeared dilute, creatinine levels were below 0.65 mg/mL (0.07 to 0.62 mg/mL), and initial absorbance readings were at least 15% lower (−15 to −26%) than the negative urine calibrator. There was very poor correlation between individual EMIT readings and THC-COOH values obtained by GC/MS

Table II. Plasma Cannabinoid Levels in ng/mL

		Time Post-Smoking (min)										
Subject	**Cannabinoid**	**5**	**10**	**20**	**30**	**50**	**65**	**120**	**180**	**240**	**300**	**1320**
	THC	57.7	56.7	43.2	41.0	23.6	13.4	12.6	5.7	3.2	2.1	ND*
1	11-OH-THC	2.0	5.7	15.1	10.3	10.3	5.5	4.5	3.2	3.0	1.0	ND
	THC-COOH	3.6	4.8	12.3	15.9	16.1	10.9	9.9	5.5	5.1	2.9	1.6
	EMIT**	46.0	59.0	54.0	57.0	61.0	61.0	28.0	28.0	48.0	27.0	14.0
	THC	—†	34.3	39.4	—	24.3	20.0	16.5	6.9	4.5	2.0	ND
2***	11-OH-THC	—	2.1	7.6	—	7.2	8.2	10.3	4.7	3.9	2.6	1.2
	THC-COOH	—	7.7	13.0	—	14.1	18.2	26.0	19.6	8.3	4.1	1.9
	EMIT	—	—	—	—	—	92.0	58.0	38.0	35.0	40.0	35.0
	THC	—	—	48.8	22.0	23.0	12.4	8.9	10.8	6.7	3.2	ND
3	11-OH-THC	—	—	10.7	14.7	10.5	8.2	3.6	4.5	2.3	ND	ND
	THC-COOH	—	—	3.1	7.0	8.8	9.3	5.2	10.3	4.8	2.2	0.8
	EMIT	—	—	27.0	—	47.0	42.0	26.0	25.0	35.0	34.0	9.0
	THC	70.8	55.7	67.6	39.2	21.2	9.8	4.2	1.9	ND	ND	ND
4	11-OH-THC	11.0	24.3	21.4	13.2	5.9	6.5	2.8	2.2	1.0	ND	ND
	THC-COOH	6.2	10.4	5.5	5.6	3.9	3.1	1.8	1.1	0.8	ND	ND
	EMIT	27.0	13.0	18.0	19.0	18.0	22.0	16.0	24.0	24.0	8.0	10.0
	THC	36.1	28.5	19.0	14.1	17.9	12.6	8.1	4.2	4.7	2.0	ND
5	11-OH-THC	6.0	6.0	11.1	9.5	5.4	4.5	3.0	2.4	1.5	ND	ND
	THC-COOH	0.8	1.8	2.5	10.2	5.5	5.5	3.1	2.5	5.1	2.0	1.1
	EMIT	36.0	28.0	66.0	72.0	90.0	56.0	35.0	43.0	32.0	18.0	9.0
	THC	44.9	41.2	25.1	22.9	13.6	12.2	4.9	2.8	1.4	ND	ND
6	11-OH-THC	9.3	14.0	10.9	6.3	3.4	2.4	2.2	1.2	ND	ND	ND
	THC-COOH	7.6	13.5	18.6	25.3	22.9	11.7	8.1	4.5	2.6	1.5	0.7
	EMIT	52.0	87.0	76.0	—	93.0	85.0	48.0	44.0	41.0	41.0	23.0
	THC	99.0	84.5	50.2	39.4	33.0	20.8	5.7	—	3.2	1.8	ND
7	11-OH-THC	1.9	4.3	7.4	14.0	16.3	16.9	10.5	—	3.6	ND	2.2
	THC-COOH	1.1	4.5	5.0	7.4	10.4	15.8	15.9	—	9.7	7.4	2.2
	EMIT	44.0	—	—	75.0	—	—	63.0	—	29.0	25.0	15.0
	THC	85.8	168.0	124.9	65.8	28.4	8.8	4.5	1.3	ND	ND	ND
8	11-OH-THC	7.3	61.9	40.7	34.2	29.8	15.5	7.6	2.2	ND	ND	ND
	THC-COOH	11.2	22.9	33.0	54.6	107.0	50.5	21.1	12.1	5.8	2.6	ND
	EMIT	43.0	56.0	57.0	45.0	53.0	41.0	41.0	43.0	26.0	29.0	8.0
	THC	109.1	117.3	77.4	59.2	30.2	18.5	13.0	4.9	2.6	ND	ND
9	11-OH-THC	5.5	33.9	38.0	28.8	15.7	10.7	8.8	5.1	3.4	1.6	ND
	THC-COOH	8.1	17.7	56.4	81.7	63.0	36.7	21.7	20.5	5.2	2.2	ND
	EMIT	31.0	58.0	62.0	65.0	67.0	63.0	28.0	37.0	35.0	21.0	12.0
	THC	177.2	119.7	82.7	69.0	57.7	41.2	19.2	6.1	3.0	ND	ND
10	11-OH-THC	4.1	16.5	26.5	35.9	22.8	17.4	13.2	4.5	3.4	2.2	ND
	THC-COOH	17.2	19.3	33.1	50.5	104.3	83.2	38.6	36.2	24.9	12.0	1.1
	EMIT	19.0	27.0	42.0	32.0	29.0	24.0	13.0	14.0	3.0	10.0	8.0

*ND = not detected by GC/MS.
**Total cross-reacting cannabinoids expressed in THC-COOH equivalents.
***Non-compliant subject, 37 ng/mL total cannabinoid in plasma before smoking.
†Sample not available.

(r = 0.230, n = 49). As noted for plasma, this lack of correlation may be due in part to individual differences in metabolism. The degree to which the acid metabolite is conjugated would affect the EMIT reading since the affinity of the antibody is lower for complexed than for free THC-COOH, and enzyme hydrolysis was not used in this study prior to EMIT analysis. EMIT values remained high for the period of study, while THC-COOH levels had fallen, in some cases to the limit of detection of the GC/MS method. The excretion of other cross-reacting polar metabolites such as polycarboxylic acids or hydroxylated carboxylic acids (18,30) may become predominant with increasing time since ingestion. Other investigators have reported that positive EMIT values can be seen from 4 to 10 days following a single exposure to cannabis (3). It appears quite likely that urines collected from individuals more than 1 or 2 days after cannabis use and found positive by EMIT or other immunoassay screen could have THC-COOH levels, even with hydrolysis, below the level of sensitivity for GC/MS verification. This possibility has been suggested in other investigations, where hydrolysis was not used prior to GC/MS confirmatory analysis, to explain poor correlations between immunoassay and GS/MS results (38,43).

The diOH-THC metabolite was found at the highest concentrations in the earliest urine samples from 9 of the 10 subjects. The levels fell rapidly in successive voidings, and in all but one subject (#7) this metabolite had fallen below the limit of detection by 22 hr. No diOH-THC was seen in urines from subject #6. These urines were also lowest for THC-COOH and the most dilute, with creatinine levels below 0.33 mg/mL. The diOH metabolite, if present, was below the limit of detection of 800 pg/mL by GC/MS. The correlation between the log metabolite ratio [THC-COOH:diOH-THC] and time post-smoking for the 26 urines in which both metabolites were measurable is shown in Figure 5. Variability in this function was too great to be suitable for interpreting time since ingestion. As with THC in plasma, the best indicator of recent use in urine was the level of the diOH-THC metabolite itself. Its presence at concentrations greater than 15 to 20 ng/mL would indicate cannabis use

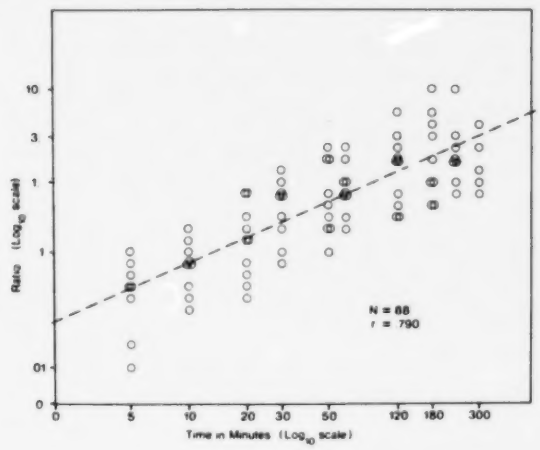

Figure 3. Correlation between plasma metabolite ratio THC-COOH/THC and time since smoking.

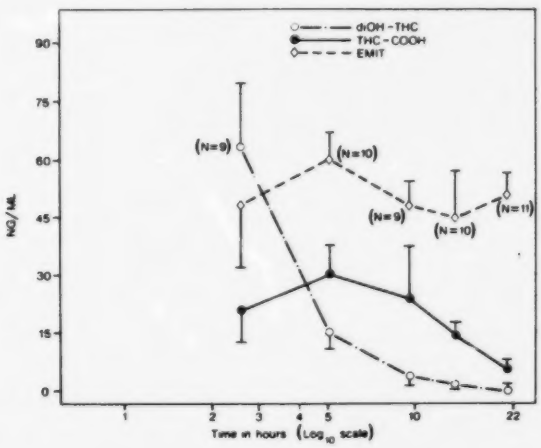

Figure 4. Mean urine cannabinoid levels ± SEM by GC/MS and EMIT.

Table III. Urine Cannabinoid Levels in ng/mL

Subject	Cannabinoid	Time Post-Smoking (hr) 0-4 (2.6)*	4-6 (5.1)	6-12 (9.4)	12-18 (13.9)	18-22 (20.7)
1	THC-9-COOH	11.7	42.6	—	23.5	19.0
	8,11-diOH-THC	82.8	47.5	—	8.5	0.0
	EMIT (Total)	62.0	83.0	—	96.0	70.0
2**	THC-9-COOH	38.6	10.0	17.8	19.8	4.7
	8,11-diOH-THC	168.5	9.0	6.1	0.0	0.0
	EMIT	97.0	44.0	70.0	78.0	87.0
3	THC-9-COOH	34.5	30.6	—	3.8	6.0
	8,11-diOH-THC	78.5	10.1	—	0.9	0.0
	EMIT	107.0	74.0	—	0.0	56.0
4	THC-9-COOH	0.8	27.6	16.5	—	3.0
	8,11-diOH-THC	34.8	12.2	0.0	—	0.0
	EMIT	4.0	76.0	40.0	—	22.0
5	THC-9-COOH	0.5	14.3	9.0	—	11.0
	8,11-diOH-THC	19.8	10.5	0.0	—	0.0
	EMIT	0.0	21.0	15.0	—	45.0
6	THC-9-COOH	1.6	2.6	5.8	4.6	2.5/0.8***
	8,11-diOH-THC	0.0	0.0	0.0	0.0	0.0/0.0
	EMIT	14.0	20.0	51.0	61.0	55.0/47.0
7	THC-9-COOH	—	4.7	11.8	16.4	8.6
	8,11-diOH-THC	—	11.1	16.1	8.7	6.2
	EMIT	—	49.0	43.0	5.0	51.0
8	THC-9-COOH	5.2	14.0	9.6/6.5	6.6	4.9
	8,11-diOH-THC	102.7	18.0	1.9/3.2	0.0	0.0
	EMIT	53.0	91.0	70.0/71.0	58.0	47.0
9	THC-9-COOH	53.8	64.1	12.9	7.4/1.8	0.0
	8,11-diOH-THC	37.2	7.9	0.0	0.0/0.0	0.0
	EMIT	82.0	73.0	35.0	59.0/64.0	57.0
10	THC-9-COOH	29.1	82.3	128.5	45.6/6.2	0.9
	8,11-diOH-THC	40.7	21.5	8.6	0.0/0.0	0.0
	EMIT	0.0	58.0	34.0	42.0/0.0	22.0

*Mean urine collection time.
**Non-compliant subject, 9.9 ng/mL THC-COOH in urine before smoking.
***Two voidings during time period.

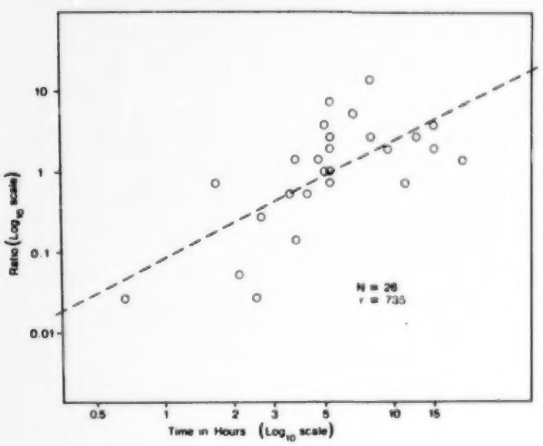

Figure 5. Correlation between urinary metabolite ratio THC-COOH:diOH-THC and time since smoking.

within the previous 4 to 6 hr and would coincide with the period of most pronounced pharmacological activity and psychomotor performance decrement (9,20,34).

Conclusion

Recent smoking, i.e. within 6 hr, is indicated by THC levels in plasma in excess of 2 to 3 ng/mL and 8β,11-diOH-THC levels in urine in excess of 15 to 20 ng/mL.

References

1. J.S. Cridland, D. Rottanburg, and A.H. Robins. Apparent half-life of excretion of cannabinoids in man. *Hum. Toxicol.* **2:** 641-44 (1983).
2. C.A. Dackis, A.L.C. Pottash, W. Annitto, and M.S. Gold. Persistence of urinary marijuana levels after supervised abstinence. *Am. J. Psychiatry* **139:** 1196-98 (1982).
3. M.J. DeLaurentis, K. McNeil, A.J. Mann, S. Clark, and H.M. Greenwood. An EMIT assay for cannabinoid metabolites in urine. In *The Analysis of Cannabinoids in Biological Fluids.* R.L. Hawks, ed. NIDA Research Monograph 42, 1982, pp. 69-84.
4. J. Irving, B. Leeb, R.L. Foltz, C.E. Cook, J.T. Bursey, and R. E. Willette. Evaluation of immunoassays for cannabinoids in urine. *J. Anal. Toxicol.* **8:** 192-96 (1984).
5. D. Rosenthal, T.M. Harvey, J.T. Bursey, D.R. Brine, and M.E. Wall. Comparison of gas chromatography mass spectrometry methods for the determination of Δ^9-tetrahydrocannabinol in plasma. *Biomed. Mass Spectrom.* **5:** 312-18 (1978).
6. J.D. Whiting and W.W. Manders. Confirmation of a tetrahydrocannabinol metabolite in urine by gas chromatography. *J. Anal. Toxicol.* **6:** 49-52 (1982).
7. V.W. Hanson, M.H. Buonarati, R.C. Baselt, N.A. Wade, C. Yep, A.A. Biasotti, V.C. Reeve, A.S. Wong, and M.W. Orbanowsky. Comparison of ^{3}H- and ^{125}I-radioimmunoassay and gas chromatography/mass spectrometry for the determination of delta-9-tetrahydrocannabinol and cannabinoids in blood and serum. *J. Anal. Toxicol.* **7:** 96-102 (1983).
8. B. Law, P.A. Mason, A.C. Moffat, and L.J. King. Confirmation of cannabis use by the analysis of delta-9-tetrahydrocannabinol metabolites in blood and urine by combined HPLC and RIA. *J. Anal. Toxicol.* **8:** 19-22 (1984).
9. B. Law, P.A. Mason, A.C. Moffat, R.I. Gleadle, and L.J. King. Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin. *J. Pharm. Pharmacol.* **36:** 289-94 (1984).
10. R.L. Foltz, K.M. McGinnis, and D.M. Chinn. Quantitative measurement of Δ^9-tetrahydrocannabinol and two major metabolites in physiological specimens using capillary column gas chromatography negative ion chemical ionization mass spectrometry. *Biomed. Mass Spectrom.* **10:** 316-23 (1983).
11. M.M. Halldin. Studies on the biotransformation of tetrahydrocannabinol in man and animals. *Acta Pharm. Suec.* **20:** 160 (1983).
12. M E. Wall and M. Perez-Reyes. The metabolism of Δ^9-tetrahydrocannabinol and related cannabinoids in man. *J. Clin. Pharmacol.* **21:** 178s-89s (1981).
13. P.L. Williams and A.C. Moffat. Identification in human urine of Δ^9-tetrahydrocannabinol-11-oic acid glucuronide: A tetrahydrocannabinol metabolite. *J. Pharm. Pharmacol.* **32:** 445-48 (1980).
14. D.E. Green. Quantitation of cannabinoids in biological specimens using probability based matching gas chromatography/mass spectrometry. In *Cannabinoid Assays in Humans.* R.E. Willette, ed. NIDA Research Monograph No. 7, 1976, pp. 70-87.
15. S.L. Kanter, L.E. Hollister, and J.U. Zamora. Marihuana metabolites in urine of man. XI. Detection of unconjugated and conjugated Δ^9-tetrahydrocannabinol-11-oic acid by thin-layer chromatography. *J. Chromatogr.* **235:** 507-12 (1982).
16. L.E. Hollister, S.L. Kanter, R.D. Board, and D.E. Green. Marihuana metabolites in urine of man. III. Unchanged delta-9-tetrahydrocannabinol. *Res. Commun. Chem. Pathol. Pharmacol.* **8:** 579-84 (1974).
17. M.M. Halldin, S. Carlsson, S.L. Kanter, M. Widman, and S. Agurell. Urinary metabolites of Δ^1-tetrahydrocannabinol in man. *Arzneim.-Forsch./Drug Res.* **32:** 764-67 (1982).
18. M.M. Halldin, L.K.R. Andersson, M. Widman, and L.E. Hollister. Further urinary metabolites of Δ^1-tetrahydrocannabinol in man. *Arzneim.-Forsch./Drug Res.* **32:** 1135-38 (1982).
19. S. Hazan, P. Beaumier, L. McBurney, and B. Bobbie. THC metabolites in urine. Proceedings of the Joint Meeting of Canadian Society of Forensic Science and The Society of Forensic Toxicologists, Toronto, 1980.
20. D.A. Attwood, L.J. McBurney, B.A. Bobbie, and M.A. Clowes. Time-course effects of moderate levels of cannabis on human psychomotor performance. In *Proceedings, 14th Annual Meeting Human Factors Association of Canada.* Toronto, 1981, pp. 19-23.
21. A. Manolis, L.J. McBurney, and B.A. Bobbie. The detection of Δ^9-tetrahydrocannabinol in the breath of human subjects. *Clin. Biochem.* **16:** 229-33 (1983).
22. J. Rosenfeld. Mass fragmentographic assays for the cannabinoids and their metabolites. In *Marihuana: Chemistry, Biochemistry, and Cellular Effects.* G.G. Nahas, ed. Springer-Verlag, Inc., New York, 1976, pp. 87-92.
23. R.L. Foltz and B.J. Hidy. Quantitative analysis for Δ^9-THC, 11-hydroxy-Δ^9-THC, and 9-carboxy-Δ^9-THC in plasma using GC/CI-MS. In *The Analysis of Cannabinoids in Biological Fluids.* NIDA Research Monograph 42, 1982, pp. 99-118.
24. EMIT-dau Cannabinoid Urine Assay. Syva Co., California, 1980, pp. 1-37.
25. R.A. Bergman, T. Lukaszewski, and S.Y.S. Wang. The detection of tetrahydrocannabinol in blood: A comparative study. *J. Anal. Chem.* **5:** 85-89 (1981).
26. H.W. Peel and B.J. Perrigo. Detection of cannabinoids in blood using EMIT. *J. Anal. Toxicol.* **5:** 165-67 (1981).
27. D.J. Harvey. The mass-spectra of the trimethylsilyl derivatives of the hydroxy and acid metabolites of Δ^1 and Δ^6- tetrahydrocannabinol. *Biomed. Mass Spectrom.* **8:** 579-88 (1981).

28. D.J. Harvey, B.R. Martin, and W.D.M. Paton. Identification and measurement of cannabinoids and their *in vivo* metabolites in liver by gas chromatography-mass spectrometry. *Adv. Biosci.* **22-23:** 45-62 (1978).
29. M.E. Wall and D.R. Brine. Identification of cannabinoids and metabolites in biological materials by combined gas-liquid chromatography-mass spectrometry. In *Marihuana: Chemistry, Biochemistry, and Cellular Effects.* G.G. Nahas, ed. Springer-Verlag, New York, 1976, pp. 51-62.
30. M.M. Halldin, M. Widman, C.V. Bahr, J-E. Lindgren, and B.R. Martin. Identification of *in vitro* metabolites of Δ^1-tetrahydrocannabinol formed by human livers. *Drug Metab. Dispos.* **10:** 297-301 (1982).
31. M.E. Wall, B.M. Sadler, D. Brine, and H. Taylor. Metabolism, disposition and pharmacokinetics of delta-9-tetrahydrocannabinol in male and female subjects. In *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects.* S. Agurell, W.L. Dewey, and R.E. Willette, eds. Academic Press, Inc., Florida, 1984, pp. 185-210.
32. J-E. Lindgren, A. Ohlsson, S. Agurell, L. Hollister, and H.K. Gillespie. Clinical effects and plasma levels of Δ^9-tetrahydrocannabinol (Δ^9-THC) in heavy and light users of cannabis. *Psychopharmacology* **74:** 208-12 (1981).
33. A. Ohlsson, J-E. Lindgren, A. Wahlen, S. Agurell, L.E. Hollister, and H.K. Gillespie. Single dose kinetics of deuterium labelled Δ^1-tetrahydrocannabinol in heavy and light cannabis users. *Biomed. Mass Spectrom.* **9:** 6-10 (1983).
34. V.C. Reeve, W.B. Robertson, J. Grant, J.R. Soares, E.M. Zimmerman, H.K. Gillespie, and L.E. Hollister. Hemolyzed blood and serum levels of delta-9-THC: Effects on the performance of road sobriety tests. *J. Forensic Sci.* **28:** 963-71 (1983).
35. J.L. Valentine, P.J. Bryant, P.L. Gutshall, O.H.M. Gan, P.D. Lovegreen, E.D. Thompson, and H.C. Niu. High-pressure liquid chromatographic-mass spectrometric determination of Δ^9-tetrahydrocannabinol in human plasma following marijuana smoking. *J. Pharm. Sci.* **66:** 1263-66 (1977).
36. A. Ohlsson, J-E. Lindgren, A. Wahlen, S. Agurell, L.E. Hollister, and H.K. Gillespie. Plasma delta-9-tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. *Clin. Pharmacol. Ther.* **28:** 409-16 (1980).
37. M. Perez-Reyes, S. Di Guiseppi, K.H. Davis, V.H. Schindler, and C.E. Cook. Comparison of effects of marihuana cigarettes of three different potencies. *Clin Pharmacol. Ther.* **31:** 617-24 (1982).
38. J.E. O'Connor and T.A. Regent. EMIT cannabinoid assay: Confirmation by RIA and GC/MS. *J. Anal. Toxicol.* **5:** 168-73 (1981).
39. A.B. Jones, H.N. ElSohly, and M.A. ElSohly. Analysis of the major metabolite of Δ^9-tetrahydrocannabinol in urine. V. Cross-reactivity of selected compounds in a radioimmunoassay. *J. Anal. Toxicol.* **8:** 252-54 (1984).
40. L. Lemberger, J.L. Weiss, A.M. Watanabe, I.M. Galanter, R.J. Wyatt, and P.V. Cardon. Delta-9-tetrahydrocannabinol. Temporal correlation of the psychologic effects and blood levels after various routes of administration. *New Engl. J. Med.* **286:** 685-88 (1972).
41. B.M. Sadler, M.E. Wall, and M. Perez-Reyes. The pharmacokinetics of delta-9-tetrahydrocannabinol in man after simultaneous intravenous and oral administration. In *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects.* S. Agurell, W.L. Dewey, and R.E. Willette, eds. Academic Press, Inc., Florida, 1984, pp. 227-38.
42. J.R. Soares and S.J. Gross. Separate radioimmune measurements of body fluid Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC. *Life Sci.* **19:** 1711-18 (1976).
43. M.A. Peat, B.S. Finkle, and M.E. Deyman. Laboratory evaluation of immunoassay kits for the detection of cannabinoids in biological fluids. In *The Analysis of Cannabinoids in Biological Fluids.* R.L. Hawks, ed. NIDA Research Monograph 42, 1982, pp. 85-98.

Manuscript received July 29, 1985;
revision received December 9, 1985.